

A COMPARATIVE STUDY OF CERTAIN SPECIES
OF MARASMIUS AND COLLYBIA
IN CULTURE

JEAN D. ARNOLD

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JEAN D. ARNOLD

(WITH PLATES 35 AND 36)

INTRODUCTION

During recent years considerable attention has been devoted to the subject of sexuality in the Hymenomycetes, with the result that conditions prevailing in many species have been determined. In the Agaricaceae the genus *Coprinus* has received the most study, but species of *Hypholoma*, *Panaeolus*, *Pholiota* and other genera have also been investigated.

It is well known to students of the Agaricaceae that some of the small members of the genus *Collybia* resemble the genus *Marasmius* very closely, not only in size and general appearance, but also in the ability of the dried pileus to revive when moistened. Since little attention has been given to species of *Marasmius* or the marasmiod species of *Collybia* with regard to their development and sexual behavior, several species of *Marasmius* and *Collybia* were used in the present study. The following species received the most attention in this work: *Marasmius elongatipes* Peck, *Collybia tuberosa* Fries, *C. cirrata* Fries and the form hitherto recognized as *C. cirrata* Fries var. *Cookei* Bres. (1).

HISTORICAL

As early as 1860 there is a record of simple experiments dealing with *Collybia tuberosa*. In "The Gardeners' Chronicle and Agricultural Gazette" for that year there is a mycological note written by Berkeley (3, p. 456). *Collybia tuberosa* is referred to there as *Agaricus tuberosus*, and Berkeley points out that the tubers them-

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selves were once considered a distinct genus, and were known as *AcrospERMUM cornutum*. The last paragraph of the article reads as follows: "It may not be uninteresting to some of our readers to state that many of these tuber-like bodies will produce their true fruit if placed under proper conditions of air, light, heat, and moisture. We have ourselves shown this to be the case in one instance many years since, and Mr. Currey, who bids fair to follow in the steps of the Messrs. Tulasne in the study of Mycology, has been successful in several more or less important cases."

However, little interest was shown in the study of this group of fungi until 1918, when Mlle. Bensaude's thesis (2) was published in Paris. This outstanding piece of work on the life cycle and sexuality of the basidiomycetes aroused widespread interest. The history of subsequent investigations dealing with this general topic has been reviewed so thoroughly elsewhere (9) (14), that mention will be made here of only those studies relating to the species dealt with in this investigation.

Most of the information on hand at present concerning the species here involved is due to the investigations of Kniep (12) (13), who reported heterothallism in *Collybia cirrata* and *C. tuberosa* and the occurrence of haploid fruit bodies in the latter species.

Kniep (14, p. 413) also studied *C. cirrata* in more detail and found as the result of mating monosporous mycelia obtained from basidiospores from diploid fruit bodies, that this species was tetrapolar with regard to the segregation of its sexual factors. He also found that monosporous mycelia originating from basidiospores from two separate fruit bodies both collected from the same wooded area, were completely interfertile when paired in all possible combinations. Monosporous mycelia from the spores of each pileus, when paired with other monosporous mycelia of the same pileus, gave a clear case of tetrapolarity.

TECHNIQUE AND MEDIA USED

The original cultures obtained in the study of these species were all procured from fresh material, either from spores or from tissue from the pileus or sclerotia when these were present, using the customary precautions against contamination. When mono-

sporous cultures were made, Kauffman's method (11) for the isolation of single spores by spraying on agar plates was used, with the following modification: Each time a plate was sprayed, half the suspension in the capsule was poured out and the volume made up again using sterile distilled water. This process was repeated several times, thereby making a series of dilutions among which a plate was obtained in which the spores were sufficiently scattered so that single spores could be isolated with little difficulty.

For cutting out the spores, a nichrome wire inoculating needle, flattened and pointed so that it resembled a minute scalpel, proved very serviceable. As each spore was cut out it was transferred to a sterile agar plate. After about two weeks' growth, the cultures were examined for clamp connections. If none appeared, transfers were made to malt agar slants. All cultures were stored on shelves in diffuse light at a temperature of about 20° C.

At first Kauffman's nutrient agar as given by Lohman (16) was used in the spore germination tests. Although it was found to be a satisfactory medium, it was later given up in favor of plain malt extract agar, which gave excellent development and was more easily prepared. The following proportions were used in the preparation of the malt agar:

Malt extract.....	25 grams
Agar.....	20 grams
Distilled water to make.....	1 liter

This was sterilized in an autoclave for 25 minutes at 12 pounds pressure. If the malt extract is completely dissolved in the water before being placed in the autoclave, and if a fairly good grade of agar is used, filtering is not necessary for ordinary purposes. Other media used were: Kotila's agar (15), Poole's medium for developing sporophores in *Collybia dryophila* (20), Etter's medium (8) and certain modifications of the last two. The following combinations were found to be the most useful for inducing fruiting in the species of *Marasmius*:

Medium 5.

Powdered oak wood.....	40 grams
Malt extract.....	10 grams
Peptone.....	0.1 gram
Water.....	120 cc.

Medium 10.

Corn meal.....	50 grams
Agar.....	15 grams
Prune extract.....	1 liter
(75 g. prunes in 1 l. water)	

The media used for the growth and fruiting of the *Collybia* species required different constituents. On Kauffman's and Kotilla's agars their growth was slow and scanty. However, since *Collybia tuberosa* and *C. cirrata* both grow upon decaying fungous material, it seemed logical to incorporate such material into the media used for their culture. In the vicinity of Ann Arbor, Michigan, *C. cirrata* grows in great abundance upon shrivelled remains of *Boletus luteus*. Accordingly, large numbers of these polypores were gathered in the autumn and dried. When needed, the dried *Boletus* caps were cooked in a little water and used as a substratum. This was placed in glass capsules 10 cm. in diameter and 7 cm. deep, and sterilized in an autoclave for 20 minutes at 15 pounds pressure. All three species of *Collybia* fruited well on this medium, producing fruit bodies (PLATE 36, FIGS. 8, 9). A decoction was also made by gently boiling for about an hour some of the dried caps in a quantity of water just sufficient to float them. To each liter of the filtrate 15 grams of agar were added. The mycelia of the three species of *Collybia* grew moderately well on this medium (PLATE 36, FIG. 1). A modification of Kauffman's agar was also used. This included all the nutrient materials as given in Kauffman's formula (11), but *Boletus* extract was used instead of water. The fungi grew fairly readily upon this, but fruiting was reduced (PLATE 36, FIG. 2 AND 3). This medium was used in small Erlenmeyer flasks, and the cultures were grown both in the dark and in diffuse light. Various media using prunes or prune extract were tried, but none of the three species of *Collybia* would grow at all on any medium containing prune. Similar results were experienced by Miss Mounce (17) with *Fomes pinicola*. The medium which was found to be most satisfactory was discovered in an attempt to duplicate as nearly as possible, under aseptic conditions, the natural habitat of the fungi. This was called medium 19 and was prepared as follows:

Ordinary greenhouse soil was placed in the bottom of glass capsules to a depth of 1.5–2 cm. and saturated with water. A dried pileus of *Boletus luteus* (or several, if small) was then laid on top of the soil, and the glass cover placed on the capsule. No cotton was used with the glass cover, as it was found that the fungi thrive better with freer access of air. The capsules and contents were then sterilized in the autoclave at 15 pounds pressure for 30 minutes, and when cool were inoculated. All three species of *Collybia* grew well on this medium and produced fruit bodies generally a little larger than those found in nature. This medium was also used in flasks with fair success (PLATE 36, FIG. 7). It was found that when the soil was sterilized first in a dry oven at a temperature of 140–150° C., and the *Boletus* and water added later and subsequently sterilized in the autoclave as above mentioned, no growth of the fungi occurred.

RESULTS

GERMINATION OF BASIDIOSPORES. Good spore germination was obtained on Kauffman's nutrient agar and on plain malt extract agar in petri dishes at room temperature (approximately 20–21° C.) in the following species: *Marasmius alliatus* (Schaeff.) Schröt., *M. capillaris* Morg., *M. elongatipes* Peck., *M. epiphyllus* Fries, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries. *Marasmius elongatipes* and the three species of *Collybia* fruited more readily than the other species, with the result that these species were studied in greater detail.

HAPLOID AND DIPLOID MYCELIA; OIDIA. In *Marasmius elongatipes* it was somewhat difficult to recognize any macroscopic difference between haploid and diploid mycelia. As a rule, the haploid mycelia were flatter, lying closer to the agar than the diploid mycelia, which were more fluffy. These differences, however, are only relative. There was a decided difference in the rate of growth, the diploid mycelium sometimes growing twice as fast as the haploid under identical external conditions. Different strains of the same species may show pronounced variation. Some of the haploid mycelia of *M. elongatipes* are brown and felty in appearance, and other haploid mycelia in the same species are white and fluffy. The diploid mycelia also may be either

pure white or brown. This will be discussed in greater detail later. No oidia have been observed on either the diploid or the haploid mycelia of *M. elongatipes*.

In each of the three species of *Collybia* studied it is easy to distinguish between haploid and diploid mycelia. All the mycelia are white or slightly creamy in color, but the haploids generally have a more opaque and powdery appearance than the diploids. Sometimes the haploid mycelium lies close to the agar, but at other times it is deep and fluffy, and always very powdery. The diploid mycelium differs in presenting radiating strands of a rather coarse nature, which are very conspicuous. The difference between a typical haploid and a typical diploid mycelium in this group is well shown by plate 36, figure 10. Clamp connections on all the diploid mycelia of these *Collybia* species are large and easily seen. Here also, there is a noticeable difference in the rate of growth of haploid and diploid mycelia. In the culture shown on plate 36, figure 10, the large haploid mat (three weeks old) had a radius of 1.4 cm. when inoculated at the periphery with a small piece of the complementary mycelium. During the five weeks following this inoculation, the original haploid mat grew 2.1 cm. in radius on the side away from the inoculation, showing that the haploid mycelium continued to grow at its customary rate. The diploid mycelium, during the same five weeks, extended radially to a distance of 5.5 cm., gradually encompassing the haploid. At the time the photograph was taken, the haploid mycelium retained its typical haploid appearance, was powdery with abundant oidia, and no clamp connections could be found anywhere upon that area.

Haploid mycelia originating from basidiospores of the same pileus showed great variation in the rate of growth. Some were very slow, attaining a diameter of only 2 cm. in two weeks at 20° C., while others grew to 7 cm. in diameter under the same conditions during the same period.

In all the haploid mycelia of the three species of *Collybia* studied, oidia were produced, generally in large quantities, formed by the breaking-up of the mycelium into individual cells which vary in length. In the slower growing haploid mycelia, practically the whole mycelial mass breaks up into oidia, while in

the faster growing haploids, the oidia make up a much smaller proportion of the mat. In both cases, however, the oidia are responsible for the opaque powdery appearance of the mycelium. The writer has never seen oidia on the diploid mycelia of the *Collybia* species studied.

Diploid mycelia of the three species of *Collybia* are easily distinguishable from each other. When the diploid mycelium of *C. tuberosa* is seven to ten days old, white beaks or small horns push out from the oldest part of the mat. These are accompanied by the watery exudate so characteristic of sclerotia formation (PLATE 36, FIG. 1). These horns are sclerotia, but under the conditions of culture they do not become hard and horny as in nature, and only a few take on the dark reddish brown color (PLATE 36, FIG. 8). The dark colored sclerotia do not produce fruit bodies as quickly as those which remain light colored. In the field, the sclerotia are clearly delimited from the stipe. In culture the sclerotium and stipe are not distinct from each other. Some of the sclerotia in culture show a rudimentary pileus at a very early age, without a well differentiated stipe (PLATE 36, FIG. 8). Later the sclerotium elongates and the pileus expands (PLATE 36, FIG. 7). These sclerotial "horns" or "beaks" are very sensitive to light, bending directly toward it (PLATE 36, FIG. 8). When the sclerotia-like bodies are injured or cut, there is a marked tendency to proliferate.

The diploid mycelium of *C. cirrata* differs from those of the other two species in having no sclerotium. It is a whitish mouldy growth with no special feature of identification (PLATE 36, FIG. 3).

C. cirrata var. *Cookei* is identified by its large irregular yellow sclerotia which soon form on the diploid mycelium. A large sclerotium or group of sclerotia develops in the oldest part of the mat first (PLATE 36, FIG. 2), and later as the mycelium extends, a ring of sclerotia may form at some distance out from the center. Large drops of watery exudate collect around the developing sclerotia (PLATE 36, FIG. 6 AND 10).

Sclerotia are also produced from haploid mycelia of *C. cirrata* var. *Cookei*, but in *C. tuberosa* no sclerotia have been seen on the haploid mycelia, although there are frequently drops of water to

be seen in the central part of the haploid mycelia, suggesting an attempt to form sclerotia.

EFFECT OF TEMPERATURE ON GROWTH. A study was made with regard to the effect of temperature on the rate of growth of diploid mycelia of four species of *Marasmius* and three of *Collybia*. These were grown in petri dishes in triplicate, using Kauffman's nutrient agar. The cultures were grown at nine different temperatures. The results are given in the following table:

TABLE I

AVERAGE GROWTH IN MM. OF DIPLOID MYCELIA OF SPECIES OF *Marasmius* AND *Collybia* AT END OF TENTH DAY AT VARIOUS TEMPERATURES

Species	Temperatures in Degrees Centigrade								
	3	5	10	14	20	25	29	35	40
<i>M. alliatus</i>	0	0	13	18	37	38	42	18	0
<i>M. capillaris</i>	0	1	13	34	48	52	0	0	0
<i>M. elongatipes</i>	2	6	35	43	58	41	7	0	0
<i>M. epiphyllus</i>	8	14	36	48	65	46	1	0	0
<i>C. cirrata</i>	2	4	0	7	23	19	0	0	0
<i>C. cirrata</i> var. <i>Cookei</i>	5	14	17	23	39	21	0	0	0
<i>C. tuberosa</i>	4	8	11	16	33	15	0	0	0

From this table it is evident that there is a decided difference in the reaction of the species to temperature. The lower temperatures inhibited the growth of *M. alliatus* and *M. capillaris* much more than *M. elongatipes*, *M. epiphyllus* and the three species of *Collybia*. *M. alliatus* was outstanding for its ability to develop at the higher temperatures. For *M. elongatipes*, *M. epiphyllus*, *C. cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa*, the optimum temperature lies at about 20° C., whereas for *M. capillaris* it is probably between 20° C. and 25° C. The sudden cessation of growth between 25° C. and 29° C. leads one to believe that perhaps the optimum lies somewhat below 25° C. *M. alliatus* has a rather high optimum temperature, probably somewhere between 25° C. and 29° C., judging from the rapid decrease in the amount of growth at temperatures above 29° C. Compared at their respective optimum temperatures, *M. epiphyllus* was the most rapidly growing species.

The rate of growth of each of the species of *Collybia* appears to be quite distinct, with *C. cirrata* var. *Cookei* growing most

rapidly of the three, while *C. cirrata* grew most slowly. For some reason the three cultures of *C. cirrata* at 10° C. failed to grow, otherwise all three of the species of *Collybia* show a steady increase in growth as the temperature increases to the optimum 20° C.

SEXUALITY. The sexual reactions of the fungi were studied in the usual way, *i.e.*, by pairing monosporous mycelia from basidiospores of a single pileus in all possible combinations. The results will be discussed by species.

1. *Marasmius elongatipes* Peck. When monosporous cultures from a single pileus of this species are paired in all possible combinations, three types of reactions are distinguishable. First, a complete mingling of two mycelia (*a*) when of the same sex, originally derived from the same basidiospore, or, in other words, where the genetic constitution is exactly the same in each mycelium; (*b*) when two sexually compatible mycelia are paired.

Second, a tendency for the two mycelia not to mingle but to remain separated from each other by a line of demarcation of greater or less degree. This reaction appeared between any two incompatible mycelia of different sexual groups (PLATE 35, FIG. 2, 3 AND 5), and it was also found to occur between two mycelia of the same sex which originated from different basidiospores (PLATE 35, FIG. 4). Sometimes the line of demarcation appears as a clear zone, and sometimes as a heaping up of mycelium, as in the last mentioned illustration. The writer has not been able to detect any correlation between this "aversion" and any particular sexual factor.

The third type of reaction is a general diploidization of the two mycelia when they are of complementary sexual constitution (PLATE 35, FIG. 6). Here there is a complete mingling, as mentioned above. A diploid mycelium in this species may be brown and felty or white and fluffy. This apparently depends upon the nature of the haploids which unite. Some of the haploid mycelia are brown, and some are pure white. A study of this color factor has shown that wherever two incompatible mycelia are paired, one brown and one white, there is no mingling of the two. Also, wherever a white haploid mycelium is paired with a brown haploid of complementary sex, the resulting diploid mycelium is brown. If two white haploid mycelia of opposite sex are

paired, the resulting diploid mycelium is white. Brown and white mycelia are found in each of the sexual groups. This offers interesting possibilities for a genetical study.

Marasmius elongatipes Peck, collection 2. This collection was made in the vicinity of Ann Arbor, Michigan, about Oct. 1, 1931, by E. B. and E. E. Mains (31-897). Monosporous mycelia were obtained (using fresh material) from two different pilei. In the first case 15 spores were isolated, and in the second case 11 were obtained. Within each group pairings were made in every possible combination, with results as given in tables II and III.

TABLE II

Marasmius elongatipes PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 1

		AB					Ab			aB				ab		
		1	6	7	8	14	15	5	17	12	16	18	19	3	4	9
AB	1	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	6	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	7	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	8	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	14	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
	15	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Ab	5	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
	17	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
aB	12	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
	19	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
ab	3	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	9	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-

+, clamp connections formed

-, no clamp connections formed

The monosporous mycelia of pileus 2 were tested against monosporous mycelia 1, 5, 12 and 3 from pileus 1, in order to assign them to their proper sexual group. The species is clearly tetrapolar in the arrangement of its sexual factors. Of the total 26 haploid mycelia, seven had the genetic constitution AB, eight were Ab, five were aB and six were ab.

Marasmius elongatipes Peck, collection 32. This group of fruit bodies was collected in Saginaw Forest, Ann Arbor, Michigan, on Nov. 20, 1931, by E. B. and E. E. Mains (31-898).

TABLE III

Marasmius elongatipes PECK. RESULTS OF PAIRING 11 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 2

		AB		Ab				aB		ab		
		33	21	25	27	31	34	35	28	20	23	32
AB	33	—	—	—	—	—	—	—	—	+	+	+
	21	—	—	—	—	—	—	—	+	—	—	—
	25	—	—	—	—	—	—	—	+	—	—	—
Ab	27	—	—	—	—	—	—	—	+	—	—	—
	31	—	—	—	—	—	—	—	+	—	—	—
	34	—	—	—	—	—	—	—	+	—	—	—
	35	—	—	—	—	—	—	—	+	—	—	—
aB	28	—	+	+	+	+	+	+	—	—	—	—
ab	20	+	—	—	—	—	—	—	—	—	—	—
	23	+	—	—	—	—	—	—	—	—	—	—
	32	+	—	—	—	—	—	—	—	—	—	—

Fifteen spores were isolated from one of the pilei while fresh, and the monosporous mycelia obtained from them were paired in all possible combinations, with results as given in table IV.

This table also shows the species to be tetrapolar. The twenty-six monosporous mycelia obtained from collection 2 were paired with four tester mycelia from collection 32, namely mycelia 7, 15, 26 and 2. Of the 104 pairings, 103 showed clamp connections. The other culture became contaminated. These results compare well with similar data submitted by Hanna (10) for *Coprinus lagopus* where complete interfertility was the rule in such crossings.

Pennington (19) gives the size of the basidiospores in this species as $7-8 \times 3.5 \mu$. Collection 63, made by the writer at Arms Lake, Michigan, Oct. 27, 1932, had spores which measured $8.9-9.9 (10.5) \mu \times 3.3-3.6 \mu$ when selected at random. The basidia bore four spores, so the larger spore size cannot be attributed to the number of spores produced as has been found by Smith (21) for two-spored forms of *Mycena*.

TABLE IV

Marasmius elongatipes PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM ONE PILEUS OF COLLECTION 32

		AB					Ab					aB			ab	
		1	3	7	14	19	4	13	15	17	20	18	25	26	2	12
AB	1	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
	3	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
	7	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
	14	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
	19	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
Ab	4	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—
	13	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—
	15	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—
	17	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—
	20	—	—	—	—	—	—	—	—	—	—	+	+	+	—	—
aB	18	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—
	25	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—
	26	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—
ab	2	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
	12	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—

This was such a striking deviation from the published size that it was considered desirable to pair monosporous mycelia of the long-spored form with monosporous mycelia of collections 2 and 32, both of which had spores agreeing very well with the size given by Pennington. Unfortunately, only one of the original monosporous mycelia from collection 63 survived in culture, so that it became necessary to hunt the long-spored form anew in the fall of 1933. As collection after collection was brought in and measured, it appeared that there were all variations in basidiospore size ranging from the size given by Pennington up to that found by the writer in the collection which came from Arms Lake. In order to study this variation, several collections of *M. elongatipes* were made in close proximity to each other at Cascade Glen, near Ann Arbor, Michigan, on Oct. 23, 1933. Mature pilei were selected from these collections and allowed to deposit their spores in sterile capsules. The pilei were selected from groups growing within a few feet or at most a few yards of each other, and were labelled A, B, C, D and E respectively. Twenty spores

were selected at random from a spore print from each of the pilei, with results as given in table V.

TABLE V

Marasmius elongatipes PECK. SHOWING VARIATION IN LENGTH OF BASIDIO-SPORES FROM FIVE COLLECTIONS OF THE SAME SPECIES

Pileus	Range of length of spores	Average length
A	7.6- 9.0	8.3
B	7.6- 9.6	8.6
C	5.6- 8.5	7.0
D	7.4-11.2	9.3
E	7.6-10.1	8.8

From these figures it is seen that the two populations C and D are extremes, each occupying a distinct range which only slightly overlap each other. The other populations occupy a range slightly intermediate between those of C and D.

The sexual reaction of the original long-spored form (collection 63) from Arms Lake was determined by mating 8 monosporous mycelia in the usual way. The results of these pairings are given in table VI.

TABLE VI

Marasmius elongatipes PECK. RESULTS OF PAIRING 8 MONOSPOROUS MYCELIA OF THE LONG-SPORED FORM, COLLECTION 63

	AB		Ab				aB	
	6	9	1	3	13	14	4	7
AB {	6	—	—	—	—	—	—	—
	9	—	—	—	—	—	—	—
Ab {	1	—	—	—	—	—	+	+
	3	—	—	—	—	—	+	+
	13	—	—	—	—	—	+	+
	14	—	—	—	—	—	+	+
aB {	4	—	—	+	+	+	+	—
	7	—	—	+	+	+	+	—

The long-spored form exhibits the tetrapolar arrangement of sexual factors as does the short-spored form, although in the eight spores isolated in this instance, only three of the sexual groups are represented.

Unfortunately only one of these monosporous mycelia survived, but it was paired with eight monosporous mycelia from each of the short-spored collections 2 and 32, and showed complete fertility with all of them. The eight monosporous mycelia from collections 2 and 32 contained two representatives of each of the four sexual groups in each case. See tables VII and VIII.

TABLE VII

Marasmius elongatipes PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4 (GENETIC CONSTITUTION AB) OF THE LONG-SPORED COLLECTION 63 WITH 8 HAPLOID MYCELIA OF SHORT-SPORED COLLECTION 2

	AB		Ab		aB		ab	
$\begin{smallmatrix} 2 \\ 63 \end{smallmatrix}$	1	6	5	21	12	16	3	4
4	+	+	+	+	+	+	+	+

TABLE VIII

Marasmius elongatipes PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4 (GENETIC CONSTITUTION AB) OF THE LONG-SPORED COLLECTION 63 WITH 8 HAPLOID MYCELIA OF SHORT-SPORED COLLECTION 32

	AB		Ab		aB		ab	
$\begin{smallmatrix} 32 \\ 63 \end{smallmatrix}$	1	3	4	13	18	25	2	12
4	+	+	+	+	+	+	+	+

Complete fertility was also found when nine monosporous mycelia of known sexual reaction, from the long-spored population D, were paired with four haploid mycelia of collection 2 and four of collection 32. The results of these pairings are given in tables IX and X.

From these data it is seen that the long-spored forms behave like the other collections of this species. The description of the species should be amended to read "basidiospores very variable as to length, 6.6–11, averaging 7–9.7 by 3.3–3.6 in width."

As has been pointed out above, the situation in this species is comparable to that in *Coprinus lagopus*, where Hanna (10) discovered that between different strains of the fungus there is complete fertility.

2. *Collybia cirrata* FRIES. The material for this study was collected by the writer in Saginaw Forest, Ann Arbor, Michigan, Oct. 1, 1932 (3220). In this region it was quite abundant, grow-

TABLE IX

Marasmius elongatipes PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 2

TABLE X

Marasmius elongatipes PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 32

TABLE IX

		AB		Ab		
		D ²	1	6	5	21
AB	1	+	+	+	+	+
	5	+	+	+	+	+
	6	+	+	+	+	+
	7	+	+	+	+	+
	9	+	+	+	+	+
Ab	3	+	+	+	+	+
	8	+	+	+	+	+
ab	2	+	+	+	+	+
	4	+	+	+	+	+

TABLE X

		AB		Ab		
		D ³²	1	3	4	13
AB	1	+	+	+	+	+
	5	+	+	+	+	+
	6	+	+	+	+	+
	7	+	+	+	+	+
	9	+	+	+	+	+
Ab	3	+	+	+	+	+
	8	+	+	+	+	+
ab	2	+	+	+	+	+
	4	+	+	+	+	+

ing amongst pine needles on the withered remains of *Boletus luteus*. This species of *Collybia* does not develop sclerotia. The base of the stipe is provided with very characteristic strigose hairs, and is distinctly rooting.

At first, only eight monosporous mycelia were obtained from this species, and they were paired together to ascertain the sexual conditions. The results of these pairings are given in table XI.

TABLE XI

Collybia cirrata FRIES. RESULTS OBTAINED BY PAIRING 8 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

		AB			Ab			ab	
		1	4	7	2	6	8	3	5
AB	1	-	-	-	-	-	-	+	+
	4	-	-	-	-	-	-	+	+
	7	-	-	-	-	-	-	+	+
Ab	2	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-
ab	3	+	+	+	-	-	-	-	-
	5	+	+	+	-	-	-	-	-

Since the reaction showed a clear case of tetrapolarity in which the sexual group aB was not represented, 23 additional spores were isolated and tested against three of these mycelia showing different sexual reaction. These results are given in table XII.

TABLE XII

Collybia cirrata FRIES. DETERMINATION OF SEX OF 23 ADDITIONAL MONOSPOROUS MYCELIA

		AB					Ab		aB					ab										
		9	13	27	33	42	17	40	11	18	21	23	29	31	20	22	25	26	37	38	39	43	44	45
AB	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Ab	2	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
ab	3	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Combining the two groups of monosporous mycelia isolated, it was found that of the total 31 mycelia, 8 had the genetic constitution AB, 5 were Ab, 6 were aB and 12 were ab.

3. *Collybia cirrata* Fries var. *Cookei* Bres. This form was collected by the writer upon humus in the woods near Duck Lake, Pinckney, Michigan, Sept. 24, 1932 (321). It has also been collected by A. H. Smith, who found it in the vicinity of Ann Arbor, Michigan, Oct. 6, 1932, growing upon soil, and by E. B. Mains, who found some on soil and some on agaric material at Lakeland, Michigan, Oct. 15, 1932 (32-879). In all cases the form is readily identified by the presence of a yellow nodular sclerotium at the base of the stipe. Seventeen monosporous mycelia were obtained and paired with each other as illustrated in table XIII.

In this case the inhibition factor or factors do not appear to be connected with any of the factors for sex determination, and lines of demarcation were frequently found between mycelia of the same sex but originating from different spores.

The proportions of the different sexual groups being so unequal in this experiment, 13 more spores were isolated and their mycelia tested as to sex. The results are given in table XIV.

Thus, in the 30 monosporous mycelia isolated altogether for this species, 5 had the constitution AB, 10 were Ab, 9 were aB and 6 were ab, giving another example of tetrapolarity.

figure 6, while the third type, the diploidization reaction, is illustrated in figure 4 of the same plate, and presents a very different picture to that found in *Marasmius elongatipes*.

4. *Collybia tuberosa* Fries. Specimens of this species were collected in the summer of 1932 on decayed agaric material in a swamp at Rock River, Michigan, by E. B. and E. E. Mains (32-184). That fall, it was also collected by the writer at Oscoda, Michigan (3266), where it was growing upon decayed agaric material and also amongst pine needles where decayed agaric

TABLE XV

Collybia tuberosa FRIES. RESULTS OF PAIRING 17 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

		AB						Ab			aB			ab					
		1	4	9	13	15	22	5	18	27	10	16	21	23	25	28	29	31	
AB	1	—	—	—	—	—	—	—	—	—	+	+	+	c	+	†	+	c	
	4	—	c	—	*	—	—	—	—	—	+	c	†	†	†	†	†	†	
	9	—	—	—	—	—	—	—	—	—	†	†	†	†	†	†	†	†	
	13	—	*	—	—	—	—	—	—	—	†	†	†	†	†	†	†	†	
	15	—	—	—	—	—	—	—	—	—	†	†	†	†	†	†	†	†	
	22	—	—	—	—	—	—	—	—	—	†	†	†	†	†	†	†	†	
Ab	5	—	—	—	—	—	—	—	—	†	—	—	—	—	—	—	—	—	
	18	—	—	—	—	—	—	—	—	†	—	—	—	—	—	—	—	—	
aB	27	—	—	—	—	—	—	†	†	—	—	—	—	—	—	—	—	—	
ab	10	†	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	16	†	c	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	21	†	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	23	c	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	25	†	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	28	†	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	29	†	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	
	31	c	†	†	†	†	†	—	—	—	—	—	—	—	—	—	—	—	

* Clamp connections began to form but did not completely fuse. Appeared as small curved branches only occasionally bridging over septum to touch neighboring cell, presumably "Pseudoschnallen" as described by Bruns-wik (5).

† These exceptions were very carefully checked, but no clamp connections were found. The apparent sterility in these cases can not be due to physiological immaturity of the mycelia, as they form clamp connections with other complementary mycelia. It may be due to an insufficient difference in the sexual "realisateurs" of Wettstein as Vandendries (23) suggests in similar instances.

material was not evident. The most characteristic feature of this species is its small dark reddish brown sclerotium, which is shaped somewhat like an elongated and rather shrivelled apple seed. It appeared to be growing upon withered pilei of *Russula*, but it was not possible to determine whether it is limited to this genus.

The results of pairing 17 monosporous mycelia of this species are given in table XV.

ATTEMPTS TO PRODUCE INTERSPECIFIC CROSSES. Since the three forms of *Collybia* used in this study appear to be closely related to each other, it seemed desirable to use the material in an attempt to obtain crosses between them. Tables XVI, XVII and XVIII embody the results of such experiments.

TABLE XVI
THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS OF *C. tuberosa* WITH THOSE OF *C. cirrata*

		<i>C. tuberosa</i>							
		AB		Ab		aB		ab	
		13	15	5	18	27	10	25	
<i>C. cirrata</i>	AB	33	—	—	—	—	—	—	—
	42	—	—	—	—	—	—	—	—
	Ab	2	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—
	aB	21	—	—	—	—	—	—	—
	23	—	—	—	—	—	—	—	—
	ab	39	—	—	—	—	—	—	—
	44	—	—	—	—	—	C	—	—

—, no clamp connections formed; c, culture contaminated

TABLE XVII
THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS OF *C. tuberosa* WITH THOSE OF *C. cirrata* VAR. *Cookei*

		<i>C. tuberosa</i>							
		AB		Ab		aB		ab	
		13	15	5	18	27	10	25	
<i>C. cirrata</i> var. <i>Cookei</i>	AB	37	—	—	—	—	—	—	—
	40	—	—	—	—	—	—	—	—
	Ab	19	C	—	—	C	—	—	—
	22	—	—	—	—	—	—	—	—
	aB	12	—	—	—	—	—	—	—
	30	—	—	—	—	—	—	—	—
	ab	35	—	—	—	—	—	—	—
	43	—	—	—	—	—	—	—	—

—, no clamp connections formed; c, culture contaminated

TABLE XVIII

THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS.
OF *C. cirrata* WITH THOSE OF *C. cirrata* VAR. *Cookei*

		<i>C. cirrata</i>							
		AB		Ab		ab		AB	
		1	4	2	6	9	18	3	5
<i>C. cirrata</i> var. <i>Cookei</i>	AB	2	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—
	Ab	19	—	—	—	—	—	—	—
	17	—	—	—	—	—	—	—	—
	ab	8	—	—	C	—	—	—	—
	9	—	—	—	—	—	—	—	—
	ab	45	—	—	—	—	—	—	—
	44	—	—	—	—	—	—	—	—

The results of the foregoing attempts to produce interspecific crosses have all been negative. Complete sterility appears to exist between these forms. Kniep (14) reported an experiment which he performed in connection with his studies upon the sex of *Collybia cirrata* Fries which is interesting in this regard. He obtained from two localities in Brandenburg (Havelberg and Finkenkrug) what he thought to be two collections of *C. cirrata*. He paired them, and found them to be completely intersterile. His statement (14, p. 414) reads as follows: "Die kleinen charakteristischen, in grossen Mengen auf verrotteten Pilzen auftretenden Fruchtkörper beiderlei Herkunft stimmten völlig miteinander überein, ebenso die Sporen und die daraus kultivierten Myzelien, die durch einen äusserst auffallenden Erdgeruch ausgezeichnet sind. Der einzige Unterschied war, dass der Pilz von Finkenkrug sehr stark zur Sklerotienbildung neigte, der andere nicht. Die Haplonten beider Pilze waren gegeneinander völlig steril."

It is evident that he was dealing with *Collybia cirrata* Fries and *C. cirrata* Fries var. *Cookei* Bres.

METHODS OF DIPLOIDIZATION. The species of fungi studied in this investigation present two distinct types of diploidization. One type is found in *Marasmius elongatipes*, the other type is exhibited by the three species of *Collybia*. In *Marasmius elongatipes* diploidization occurs in the same manner as that described by Buller (6) for *Coprinus lagopus*. In this type, when two haploid mycelia of complementary sexual constitution

are paired, the diploid condition arises first at the line of contact of the two mycelia and spreads from there through both of the original haploid mycelia, progressing more rapidly in the younger parts of these mats. It is very difficult to see any macroscopic differences between haploid and diploid mycelia of *M. elongatipes*, if one leaves out of consideration the difference in the rate of growth of haploid and diploid mycelia. The method recommended by Buller (6) for studying diploidization, namely that of inoculating a large mat of a haploid mycelium with a small piece of a complementary mycelium at the periphery, brought out clearly the difference in the two kinds of diploidization. In plate 36, figure 6, a large mat of haploid mycelium 7 of *Marasmius elongatipes*, with the genetic constitution AB, was inoculated at a point on its margin with a small piece of haploid mycelium 2, whose genetic constitution was ab. At the time the photograph was taken, two weeks later, the culture was examined and it was seen that the diploid condition had spread through much of the haploid mycelium 7. The extent of the diploid condition, as determined by the presence of clamp connections, is shown by the letter d, while the areas in which clamp connections were not found are marked h, presuming that such areas are still haploid.

The three species of *Collybia* showed a very different phenomenon from that of *Marasmius elongatipes*. In this case, when two complementary haploid mycelia were paired, the diploid mycelium formed along the line of contact, but the mycelia originally haploid remained so. The diploid mycelium grows so much faster than the haploid that it soon grows out and surrounds the latter. It is easy to tell even macroscopically which is haploid and which is diploid mycelium (PLATE 36, FIG. 4). Large haploid mycelia were inoculated with a small amount of their complementary mycelium placed at a point on their margin, and the manner of diploidization is shown on plate 36, figure 10. The haploid mycelium remains haploid and retains the opaque powdery appearance characteristic of the haploid mycelia of this group, while the diploid mycelium forms as a fan, gradually surrounding the haploid, and easily distinguishable from it by the possession of rather coarse radiating hyphae which are quite conspicuous. This might be regarded as a sort of "limited diploidization," for

there is certainly no progressive transformation of haploid into diploid mycelium in this case. This "limited diploidization" may be similar to that which Vandendries (26) has recently reported in *Trametes suaveolens*.

STUDIES CONCERNING MYCELIAL FUSIONS. Davidson, Dowding and Buller (7) made careful observations on the formation of fusions between different mycelia in their study upon dermatophytes. They found that fusions would occur only between mycelia of the same species, and were enabled thereby to identify species of dermatophytes much more quickly than before. Vandendries (28) has found that this is true also for basidiomycetes, and that all mycelia of one and the same species are capable of forming mycelial fusions with each other, even though they may be sexually incompatible haploids. It seemed advisable to subject the three forms of *Collybia* concerned in this study to such a test. Diploid mycelia of *C. cirrata* were paired with diploid mycelia of *C. cirrata* var. *Cookei* on an agar film in Van Tieghem cells as described by Davidson, Dowding and Buller (7). Similar pairings were made of diploid mycelia of *C. cirrata* and *C. tuberosa*, and of *C. cirrata* var. *Cookei* and *C. tuberosa*. Pairings were also made between two diploid mycelia of the same species in each case, e.g., *C. tuberosa* with *C. tuberosa*. There were numerous fusions observed between mycelia of the same species, and between different branches of the same mycelium, but no mycelial fusions were noted in which two mycelia of different species were involved. Neither were there any fusions between the diploid mycelia of *C. cirrata* and *C. cirrata* var. *Cookei*. In this instance, moreover, it was observed that *C. cirrata* var. *Cookei* seemed to be adversely affected when its hyphal tips penetrated into the edge of the area covered by the mycelium of *C. cirrata*, for its tip branches became distorted and gnarled, and produced several unnaturally short and stunted branchlets at the end of the hyphae. No such reaction occurred between *C. cirrata* and *C. tuberosa*, or between *C. cirrata* var. *Cookei* and *C. tuberosa*.

SPOROPORE PRODUCTION UPON ARTIFICIAL MEDIA. *Marasmius elongatipes* Peck. (PLATE 35, FIG. 1) fruited readily upon several media including malt agar, medium 5, medium 10, Kauffman's nutrient agar, and modifications of Poole's medium

and Etter's medium. On Kauffman's agar the fruit bodies were not normal, and did not produce spores. On all the other media the sporophores were practically normal, except that the stipe in some cases was thicker than it is in nature. From three of the compatible matings between collections 2 and 32 of this species, eight small sporophores were produced in the petri dishes.

Marasmius capillaris Morg. fruited upon medium 5, while *M. epiphyllus* Fries fruited upon medium 5 and medium 10.

Collybia cirrata, *C. cirrata* var. *Cookei* and *C. tuberosa* all fruited on various media which contained as a base the dried pilei of *Boletus luteus*. Although *C. tuberosa* is reported as usually growing upon remains of species of *Russula*, and *C. cirrata* var. *Cookei* generally on soil, it was interesting to see them thrive on *Boletus luteus*, the substratum on which *C. cirrata* grows in this region.

An attempt was made to grow the species of *Marasmius* upon the *Boletus* medium, but not the slightest trace of growth of these species could be obtained on this material.

None of the above-mentioned species produced fruit bodies when grown in the dark, nor have sporophores been produced by any of the haploid cultures up to the present time.

LONGEVITY AND ABILITY OF PILEI TO REVIVE. From time to time during the course of this study, attempts were made to obtain spore deposits from revived pilei of various species of *Marasmius* and *Collybia*. *Marasmius elongatipes*, *M. delectans*, *M. alliatus*, *M. urens* and *M. rotula* all gave negative results. They regained their normal shape and texture, but deposited no spores. *Collybia cirrata* var. *Cookei* and *C. tuberosa*, however, gave better results. *C. cirrata* var. *Cookei* when revived after 51 days of storage in a dry condition gave a good spore deposit when suspended over an agar plate, and *C. tuberosa*, after 86 days in a dried state, also yielded a good spore print. Moreover, the spores in both instances were viable. In each case, two out of four revived pilei deposited their spores readily.

DISCUSSION. Two different types of diploidization have been noted in these studies. Buller (5), in his work upon *Coprinus lagopus*, found that when two monosporous mycelia of complementary sexual constitution were paired, the diploid condition

formed first at the line of contact of the two mycelia and subsequently spread throughout both mycelia, advancing more rapidly through the young hyphae than through the older. This he believed due to the passage of rapidly-generated daughter nuclei of the one mycelium travelling through the other mycelium and becoming associated with the nuclei of the mycelium through which they were passing to form pairs of nuclei which thereafter would divide conjugately. As has been shown, *Marasmius elongatipes* behaves very much the same as *Coprinus lagopus*.

In the three species of *Collybia* studied, an entirely different kind of diploidization prevails. Here there is no spread of the diploid condition through or across the paired haploids. The diploid cells arise merely at the line of contact, and from them diploid hyphae develop and spread out in fans from between the two haploid mycelia. On account of its greater rapidity of growth, the diploid mycelium grows around the two haploids, but in doing so, it encroaches little, if any, upon them. In the culture shown on plate 36, figure 10, it can be seen that the haploid mycelium continued to grow at its usual rate even after the diploid mycelium had begun to develop.

While *Collybia cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa* all present this "limited diploidization," it is interesting to note that H. J. Brodie (4) has reported that *C. velutipes* Fries has a type of diploidization resembling that of *Coprinus lagopus*.

Several cases have been reported where the diploid mycelium appears to be limited to a narrow region along the line of contact. Vandendries (26) has made mention of this condition in *Trametes suaveolens*. The same author in collaboration with H. J. Brodie has recorded it also in *Polystictus versicolor*, and Smith and Brodie (22) in an article on *Pholiota polychroa* (Berk.) Smith & Brodie have pointed out the same fact. In all of these cases, however, there appears to be considerable growth of the haploids before there is any sign of diploid mycelium forming, and when it does appear, the diploid mycelium does not seem to have the greatly accelerated rate of growth that it has in the case of the *Collybia* species. In all of these cases also the authors report that the diploid mycelium grows across or over the haploid, the diploid and haploid mycelia mingling until finally there is great

difficulty in finding a trace of the original haploid mycelia. This is quite different from the situation in the three species of *Collybia* described in the present paper, although in all these cases the diploidization might be described as strictly "limited."

The variations in reactions between different haploid mycelia of a single species when paired together present perplexing questions. "Lines of demarcation," "inhibition," "Hemmungsreaktion," "aversion," "repulsion" and "barrage" are terms which have been used by different investigators to denote various types of incompatibility.

Some investigators have found in the results of their experiments very clear cut reactions. Vandendries (25) found that "barrage" was dependent on certain combinations of Mendelian factors, and traced a distinct correlation between the occurrence of "barrage" and the common possession of the *a* or *a'* factor by the two paired mycelia. He maintains (27) that this "barrage sexuel" cannot exist in bipolar species, and must not be confused with the phenomena of demarcation which do not behave in a Mendelian fashion.

In *Marasmius elongatipes* it is evident that there is something in the nature of an inhibition or demarcation factor which is not associated with the sexual factors. In fact, demarcation between mycelia in this species is more prevalent than mingling of mycelia, for complete mingling only occurred when diploidization took place or when the paired mycelia had originated from the same spore. Even mycelia of the same sexual constitution refused to mingle if they had arisen from different spores. Miss Mounce (17) found in her studies on *Fomes pinicola* that lines of demarcation were very general between monosporous (and even between diploid) mycelia, and that mingling occurred only between "closely related" mycelia. Oort (18) reported similar conditions in *Coprinus fimetarius* when he distinguished between "OO-Kombinationen" (mycelia from the same basidiospore) and "O-Kombinationen" (mycelia of the same sex but from different basidiospores), and reported that in the latter combinations he sometimes found a weak repulsion.

Marasmius elongatipes presents an unusual condition in regard to the variation in size and shape of basidiospores. The spore

size is commonly given as $7-8 \times 3.5 \mu$ (19) for this species. However, in normally deposited spore prints some spores have been found only 5.6μ in length, while others measured as much as 11.2μ in length. The width varied very slightly, ranging from 3.3μ to 3.6μ ; consequently the variation in length involved a great diversity of spore forms as well as spore dimensions. The range of size in the short-spored group slightly overlapped the range of the large-spored group, while other populations were intermediate. When mated, the sexual strains of the long-spored group were completely interfertile with those of the short-spored group, showing that they are races of the same species.

In studying the relationship of *Collybia cirrata* Fries var. *Cookei* Bres. to *C. cirrata* Fries and *C. tuberosa* Fries, all attempts to produce interspecific hybrids yielded only negative results. Microscopic examination showed that there were no mycelial fusions between these species when grown together. The diploid mycelium of *C. cirrata* var. *Cookei* became greatly branched and stunted at the tips of the hyphae when grown along with *C. cirrata*. This was the only case observed where one mycelium had a visible effect on the other. The complete absence of mycelial fusions between the three forms when paired with each other, and the fact that the production of sclerotia was a consistent feature of the diploid mycelia of *C. tuberosa* and *C. cirrata* var. *Cookei*, while sclerotia were never developed in *C. cirrata*, furnish evidence that the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.² is in reality a specific entity.

All three species of *Collybia* studied showed the same type of oidia on the haploid mycelia. No oidia were observed on diploid mycelia of these species of *Collybia*. On *Marasmius elongatipes* no oidia were produced either on the haploid or on the diploid mycelia.

The power of revival has long been considered a diagnostic character of the genus *Marasmius*. When a dried and shrivelled specimen of *Marasmius* is placed for a time in a moist chamber, there will be a marked return to the normal shape and texture. This was found true not only for the species of *Marasmius* studied, but also for the three species of *Collybia*. Spore deposits

² *Collybia Cookei* (Bres.) comb. nov. is the name proposed for the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.

were not obtained from any of the revived plants of *Marasmius*. On the other hand, revived plants of two of the species of *Collybia* deposited abundant viable spores. Whether the spores of *C. Cookei* and *C. tuberosa* were produced after revival, or whether it was merely a mechanical shedding of spores already formed, was not determined. This evidence would tend to put this group of *Collybia* species into the section Marasmioidae instead of the section Vestipedes where they have been placed for so many years.

SUMMARY

1. During the course of this investigation, the vegetative growth and sexual reactions have been studied for the following fungi: *Marasmius elongatipes* Peck, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries.

2. These were all found to be tetrapolar species.

3. Strains of *Marasmius elongatipes* from different localities in the vicinity of Ann Arbor, Michigan, were completely interfertile with each other when paired.

4. The kind of diploidization presented by *Marasmius elongatipes* was found to agree with the diploidization phenomenon as described by Buller for *Coprinus lagopus*.

5. A different type of sexual reaction was found in the *Collybia* species studied. Here diploidization is localized, being confined to the region of contact of the paired mycelia. Diploid cells form in this region, and from them the diploid mycelium grows, but the original haploid mycelia are not converted into diploid mycelia. For this, the term "limited diploidization" is suggested.

6. Oidia were found to occur on haploid mycelia of the three *Collybia* species studied but not on the haploid mycelia of *Marasmius elongatipes*. No oidia were seen on diploid mycelia of any of the species studied.

7. All attempts to produce hybrids between the three closely related forms of *Collybia* yielded only negative results.

8. The mycelial fusion test for specific identity was used with these species, and no fusions were observed between the three forms.

9. It is therefore concluded that *Collybia cirrata* Fries var. *Cookei* Bres. should be given specific rank, as *Collybia Cookei* (Bres.) J. Arnold.

10. Sporophore production was obtained upon artificial media for *Marasmius elongatipes*, *M. capillaris*, *M. epiphyllus*, *Collybia cirrata*, *C. Cookei* and *C. tuberosa*.

11. Pilei of *Marasmius* species were revived, but no spore deposits were obtained from them.

12. Pilei of *Collybia* species were successfully revived after more than two months in a dried condition. Basidiospores were discharged from them and germinated in the usual time on nutrient agar.

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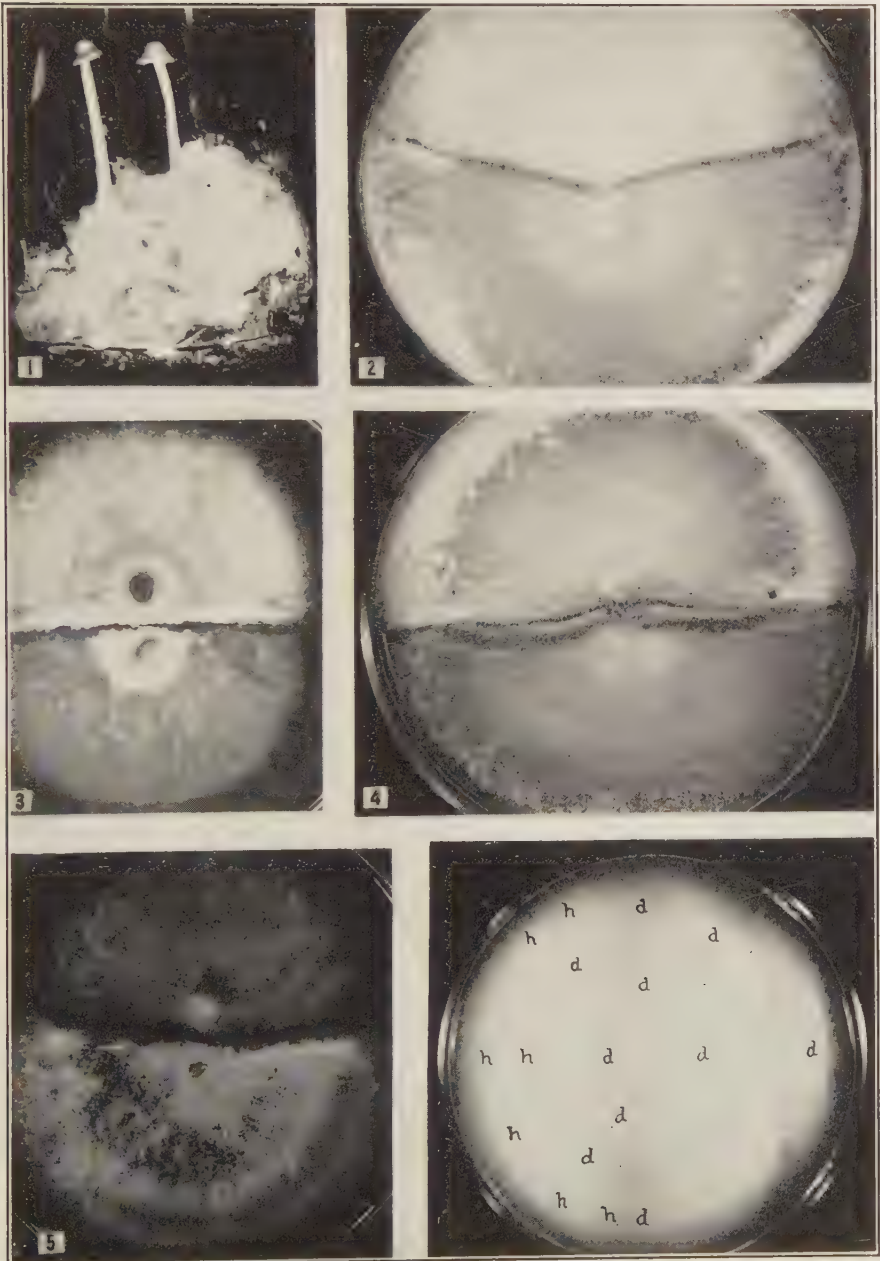
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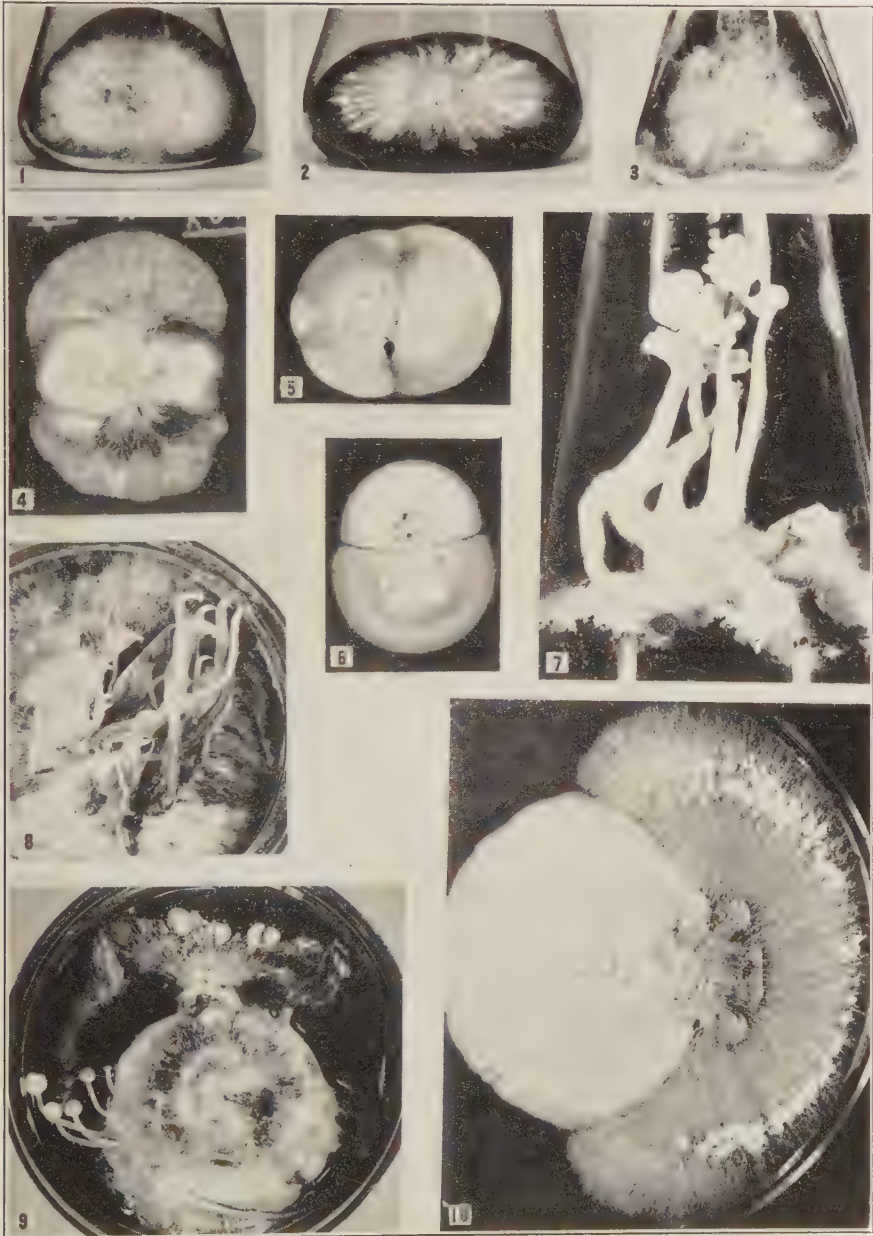
EXPLANATION OF PLATES

PLATE 35

Marasmius elongatipes Peck; 1, sporophore development on medium 5; 2, two incompatible haploid mycelia, showing the line of demarcation between them (3 ab × 16 ab); 3, two incompatible haploid mycelia, showing line of



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COLLYBIA SP.

demarcation between them (2 ab $\times$ 20 Ab); 4, two mycelia of the same sex but from different basidiospores, showing line separating the two mycelia (8 AB $\times$ 15 AB); 5, two incompatible haploid mycelia, showing line of demarcation between them (14 AB $\times$ 26 aB); 6, diploidization as it occurs when a large haploid mycelium (7 AB) is inoculated at the margin with a complementary haploid mycelium (2 ab). Areas already diploid marked d, areas still haploid marked h.

PLATE 36

Fig. 1, *Collybia tuberosa* Fries, showing the development of horn-like sclerotia on *Boletus* extract agar; 2, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.) showing the development of large irregular sclerotia on Kauffman's agar in which *Boletus* extract was used instead of water in the formula; 3, *Collybia cirrata* Fries, development on Kauffman's agar in which *Boletus* extract was used instead of water; 4-6, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 4, two haploid mycelia of complementary sexual constitution paired to show how the diploid mycelium forms "Limited diploidization" (15 aB $\times$ 17 Ab); 5, two mycelia from the same spore paired to show complete intermingling (8 aB $\times$ 8 aB); 6, two mycelia of the same sexual constitution but from two different spores paired to show the line of demarcation between them (15 aB $\times$ 16 aB); 7 and 8, *Collybia tuberosa* Fries; 7, development of abnormally large sporophores on *Boletus pilei* laid on top of moist soil; 8, showing sclerotia elongating into stipe, and pilei beginning to differentiate, also showing how sclerotia turn towards light; 9 and 10, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 9, development of sporophores on pilei of *Boletus luteus*; 10, diploidization as it occurs when a large haploid mycelium (2 AB) is inoculated at the margin with a complementary haploid (3 ab), diploid mycelium gradually growing around haploid. (Contrast with figure 6 on previous plate.)

